

Biogeographic assessment of Gorgonian-associated bacteria with antipathogenic Urinary Tract Infections (UTIs) in Karimunjawa Marine National Park, Java Sea, Indonesia

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Abstract

Gorgonian corals of Karimunjawa are impacted by anthropogenic activities, such as increasingly high mariculture intensity with consequent eutrophication, overfishing, tourism, sewage, and other pollutant discharges, which result in changes in the microbial community structure. In this study, bacterial communities associated with six species of Gorgonian, *Viminella* sp., *Ellisella* sp., *Antipathes* sp., *Melithaea* sp., *Astrogorgia* sp., and *Junceella* sp. from both the Marine Protected Area (MPA) and non-Marine Protected Area (non-MPA) zones were screened for their antipathogenic potential against Urinary Tract Infections (UTIs) pathogens. The selected bacterial isolates were identified and compared for their abundance and diversity between the two zones. A total of 156 bacterial strains were assayed for their prospective antipathogenic compounds against seven UTI pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus saprophyticus*, *Acinetobacter baumannii*, *Klebsiella pneumonia*, and *Candida albicans*. The results showed that 17 of 92 (18.48%) and 6 of 64 (9.37%) bacterial isolates from MPA and non-MPA, respectively, exhibited antimicrobial activity in at least one of the UTI pathogens. By analyzing the gene of 16S rRNA, it was discovered that the 17 isolates of MPA were associated with phyla Actinobacteria, Firmicutes and Proteobacteria, including *Streptomyces zhaozhou*, *Nocardiopsis salina*,

Micrococcus endophyticus, *Brevibacterium casei*, *Micrococcus yunnanensis*, *Saccharopolyspora coralli*, *Bacillus paramycoides*, *Virgibacillus salarius*, *Oceanobacillus iheyensis*, and *Vibrio alginolyticus*. In contrast, only six selected isolates of non-MPA were associated with the phyla Actinobacteria and Proteobacteria, including *Nocardiopsis salina*, *Micrococcus yunnanensis*, and *Acinetobacter soli*. The Diversity Index (H'), Species Richness (S), and Relative Abundance of the MPA zone were higher than those of non-MPA. These results demonstrated that Gorgonian octocoral species in the MPA region harbour varied bacteria and we propose that many Gorgonian-associated bacteria have the prospective for advancing broad-spectrum antibiotics.

Keywords

antimicrobial activity, diversity, Gorgonian-associated bacteria, Marine Protected Area, UTIs pathogens

Introduction

Karimunjawa National Park (KNP) is a mini-archipelago with 27 small islands, located in the Java Sea. This Archipelago was among the first maritime areas recognized in Indonesia as being necessary for marine biodiversity conservation. This Park is a precious and diverse tropical water ecosystem that is composed of tranquil white beaches, hard corals, soft coral, Gorgonian corals, seaweeds, mangrove, seagrass beds, birds, turtles, and many kinds of sea creatures, from crabs, anchovy, starfish, sharks, stingray, jellyfish, red snappers, etc. However, anthropogenic pressures, such as high population growth, demands for living space, the development of marine tourism, and increasing sea transport/traffic have affected marine life and its vicinity. Under the Decree of the Director-General of PHKA no. 127/Kpts/DJ-VI/1989, the Islands were zoned into 3 (three) management zones, including a marine protection area (MPA) zone, a utilization zone, and a non-marine protected (non-MPA) area (Campbell et al. 2013). The MPA includes protected areas in the sea that restrict some human activity for conservation purposes, typically to protect natural and cultural resources. The MPA has proven to effectively conserve marine biodiversity and restore ecosystem functioning (Giakoumi and Pey 2017). However, the role of MPA in providing resilience to global threats, such as biological diversity, is poorly understood. Hence, assessing the effects of MPA on biodiversity is crucial for effective MPA planning and management. This study investigated the bacterial diversity of Gorgonian-associated bacteria with antipathogenic potential UTIs in MPA and non-MPA of Karimunjawa.

In Indonesia, UTI incidence is around 180,000 new cases per year (Sugianli et al. 2020). *Escherichia coli* is the most dominant pathogen acquired nosocomially (Ejrnæs 2011). Nosocomial infections are infections acquired during the receipt of health services and may also appear after discharge from the hospital (Grasselli et al. 2017). Infection occurs when a pathogen spreads to a susceptible patient. UTIs describe microbial colonization and infection of urinary tract structures and are grouped by the site of infection as kidneys, bladder and urethra (Sheerin 2011). In this study, the

bacteria causing UTI, *Staphylococcus saprophyticus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Candida albicans* were used as test bacteria (Orhan et al. 2010; Ochada et al. 2015). The emergence of multidrug-resistant organisms (MDR) is another complication seen in nosocomial infections, a serious problem that needs to be resolved immediately in many developing countries, including Indonesia (Lee et al. 2014; Fernández et al. 2019). Therefore, it is necessary to find new antibiotics to combat the developing MDR infectious diseases.

Octocoral gorgonian is part of a family of Gorgoniidae belonging to the order Alcyonacea, subclass Anthozoans, and phylum Cnidaria (Almeida et al. 2014; Horvath 2019). This organism is ubiquitous in Karimunjawa and has been observed on some islands, from inshore to seabed (Sabdon et al. 2022). Gorgonian corals (sea fans) are characterised by their fan-like shape, providing nutrition and protection for other marine animals (Matulja et al. 2020; Sanchez et al. (2021). Gorgonian corals do not have a CaCO_3 framework for self-protection, unlike hard corals. To survive, they produce various secondary metabolites to maintain their stability (Matulja et al. 2021). However, the main obstacle faced in utilising these secondary metabolites for drugs is the problem of supply (Lindequist 2016). In contrast, marine microorganisms have recently attracted greater attention because it is known that the association of microbes with Gorgonians also synthesises the same secondary metabolites as their hosts (Modolon et al. 2020; Liu et al. 2021). Therefore, through fermentation and genetic engineering systems, microbes can supply a large number of active compounds. Performing rapid regeneration can also overcome supply problems (Wang et al. 2017). It is well known that the Gorgonian octocoral harbours many symbiotic microorganisms and produces a variety of bioactive compounds that are very important for drug discovery (Sang et al. 2019; Modolon et al. 2020). This study investigated the diversity of Gorgonian-associated bacteria with antipathogenic potential against nosocomial pathogens of UTI infections from the MPA and non-MPA zones of Karimunjawa, Java Sea.

Materials and methods

Sampling and bacterial isolation

This study was conducted at four islands across Karimunjawa National Park on March 2021, under SIMAKSI Permit no.: 1470/T.34/TU/SIMAKSI/03/2021. Sampling was carried out at the MPA zones, Burung Island (06°37'16.9"S, 110°38'07.2"E), Geleang Island (05°52'56"S, 110°21'29"E), and the non-MPA zones, Sambangan Island (06°35'08.5"S, 110°38'24.8"E) and Seruni Island (05°51'13.3"S, 110°34'36.8"E) of Karimunjawa, Java Sea, Indonesia by scuba diving (Fig. 1). Six octocoral Gorgonian *Viminella* sp., *Ellisella* sp., *Antipathes* sp., *Melithaea* sp., *Astrogorgia* sp., and *Junceella* sp. were photographed *in situ* underwater, sampled, placed in a ziplock, and brought to the laboratory (Fig. 2).

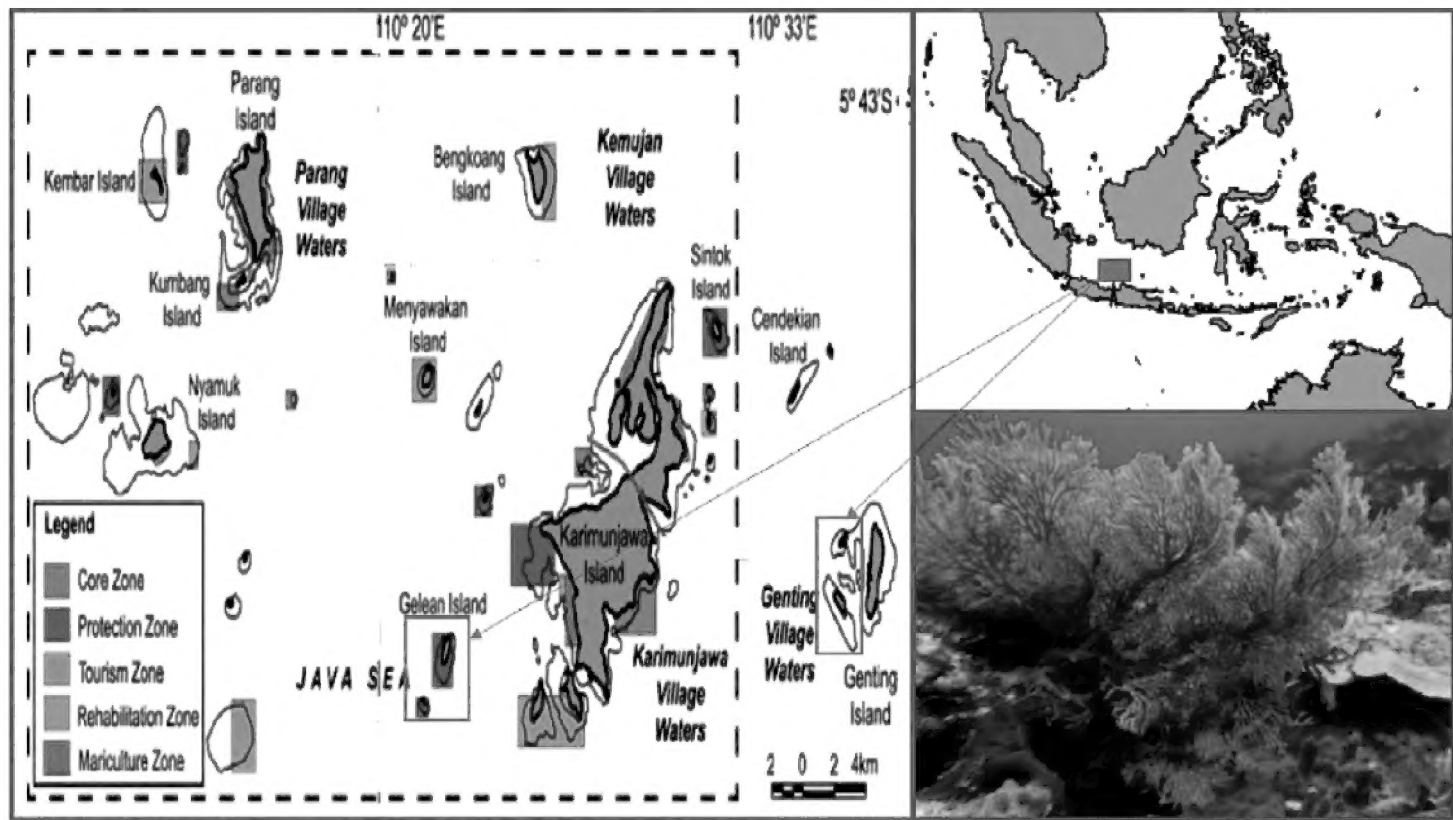


Figure 1. Map of Karimunjawa (Campbell et al. 2013).



Figure 2. Gorgonian of Karimunjawa Archipelago (Notes: **A** *Melithaea* sp. **B** *Astroorgia* sp. **C** *Antiphates*; **D** *Ellisella* sp. **E** *Junceella* sp. **F** *Viminella* sp.).

Bacterial isolation was carried out by using the serial dilution method. The sample was diluted to the concentrations of 10^{-0} , 10^{-1} and 10^{-2} ; 100 μ l of each concentration was inoculated into a Petri dish containing marine agar Zobell 2214E media. Inoculated samples were spread evenly and incubated at room temperature. After 2×24 h incubation, purification was performed by inoculating bacterial colonies using the streak method and incubating at 36 °C.

Screening of antimicrobial activity

The antimicrobial activity screening was carried out using the agar plug method against UTIs pathogens, such as *S. aureus*, *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *S. saprophyticus*, *A. baumannii* and *C. albicans*. The pure bacterial cultures were spread evenly into the Zobell medium and incubated for 3×24 hours. Pathogenic bacteria were refreshed on Nutrient Agar (NA) for 1×24 hours. Pathogens were inoculated into a test tube containing Nutrient Broth to match their density with the McFarland 0.5 standard. Pathogenic bacteria were swabbed evenly into Mueller Hinton Agar (MHA) medium, followed by placing the plugs on the media and incubated for 3×24 hours. The clear zone around the plug indicated the presence of antimicrobial activity.

16S rRNA gen-PCR amplification

PCR amplification of partial 16S rRNA gene of selected Gorgonian-associated bacteria, purification of PCR products and subsequent sequencing analysis were performed according to Wijaya et al. (2022) methods.

Data analyses

The biological indexes, such as Species Richness (SR), Relative Abundance (RA), the Shannon Diversity Index (H'), the Pielou's Evenness Index (E') and the Jaccard Index were used to analyse the data obtained in the laboratory.

Nucleotide sequence accession numbers

The accession numbers of the 16S rRNA sequences of the prospective strains were deposited in GenBank, including OL831129, OL831140, OL831143, -, OL944616, OL831230, OL832059, OL830778, OL832112, OL832113, OL824939, OL825016, OL824940, OL825006, OL825002, OL830784, OL862993, OL830811, OM108167, OM108139 and OM108168 for the isolates GL.6.5, GL.7.3, GL.7.5, GL.9.1, GL.9.2, GL.17.13, GL.17.16, GL.17.15, GL.17.21, GL.17.34, BU.2.5, BU.6.2, BU.7.3, BU.19.2, BU.20.1, SA.16.3, SA.19.2, SA.19.3, SE.10.2, SE.10.3 and SE.12.2

Results

Antimicrobial screening of Gorgonian-associated bacteria

Out of 156 isolates, 14.7% ($n = 23$) showed antimicrobial activity against UTIs pathogens (Tables 1 and 2). The total active isolates isolated from the MPA area

(18.48%) were higher than those from the non-MPA area (9.37%). Amongst 23 active isolates, three isolates can inhibit the growth of more than one pathogen, whereas the remaining isolates (n = 20) inhibited only one pathogen tested. Most of the active isolates (43.4%) were capable of inhibiting the growth of *E. coli*, followed by *P. aeruginosa* (26.1%), *S. aureus* (17.4%), *A. baumannii* (17.4%) and *K. pneumonia* (8.7%), respectively. No isolates could inhibit the growth of *S. saprophyticus* and *C. albicans*.

Table 1. Total of Gorgonian-associated bacteria and active isolates in MPA and non-MPA.

| Genus | MPA | | Non-MPA | | Σ isolate/ active |
|------------------------|-----------|-----------|-----------|----------|-------------------|
| | Σ isolate | Σ active | Σ isolate | Σ active | |
| <i>Viminella</i> sp. | 20 | 2 | 7 | 0 | 27/2 |
| <i>Ellisella</i> sp. | 27 | 8 | 19 | 1 | 46/9 |
| <i>Antipathes</i> sp. | 11 | 2 | 7 | 0 | 18/2 |
| <i>Melithaea</i> sp. | 15 | 1 | 10 | 2 | 25/3 |
| <i>Astrogorgia</i> sp. | 14 | 3 | 12 | 2 | 26/5 |
| <i>Junceella</i> sp. | 5 | 1 | 9 | 1 | 14/2 |
| Total | 92 | 17(18.5%) | 64 | 6(9.4%) | 156/23 |

Table 2. Antipathogenic assay of selected bacterial active against UTIs pathogens.

| Host | Isolate Code | Identification | Indicator Test: | | | | | | |
|------------------------|--------------|------------------------------------|-----------------|----|---|---|---|---|---|
| | | | A | B | C | D | E | F | G |
| <i>Viminella</i> sp. | GL6.5 | <i>Streptomyces zhaozhoue</i> | - | + | - | - | - | - | - |
| | GL7.3 | <i>Nocardiopsis salina</i> | + | - | + | - | - | - | - |
| <i>Ellisella</i> sp. | SA16.3 | <i>Nocardiopsis salina</i> | - | - | - | - | + | - | - |
| | GL9.1 | <i>Nocardiopsis salina</i> | - | + | - | - | - | - | - |
| | GL9.2 | <i>Nocardiopsis salina</i> | - | - | + | - | - | - | - |
| | GL17.13 | <i>Oceanobacillus iheyensis</i> | - | - | + | - | - | - | - |
| | GL17.16 | <i>Micrococcus endophyticus</i> | - | - | + | - | - | - | - |
| | GL17.15 | <i>Nocardiopsis salina</i> | - | + | - | - | - | - | - |
| | GL17.18 | <i>Kocuria palustris</i> | - | - | - | - | + | - | - |
| | GL17.21 | <i>Bacillus paramycoides</i> | + | - | - | - | - | - | - |
| <i>Junceella</i> sp. | GL17.34 | <i>Virgibacillus salarius</i> | - | + | - | - | - | - | - |
| | SA19.2 | <i>Micrococcus yunnanensis</i> | - | + | - | - | - | - | - |
| <i>Melithaea</i> sp. | SA19.3 | <i>Micrococcus yunnanensis</i> | - | - | - | - | - | + | - |
| | BU2.5 | <i>Brevibacterium casei</i> | - | + | - | - | - | - | - |
| | BU6.2 | <i>Vibrio alginolyticus</i> | - | + | - | - | - | - | - |
| <i>Antipathes</i> sp. | SE10.2 | <i>Micrococcus yunnanensis</i> | + | - | - | - | - | - | - |
| | GL14.22 | <i>Marinococcus halophilus</i> | - | + | - | - | - | - | - |
| | GL222 | <i>Micrococcus yunnanensis</i> | - | - | - | - | + | - | - |
| <i>Astrogorgia</i> sp. | BU14.4 | <i>Pseudomonas stutzeri</i> | + | + | - | - | - | - | - |
| | BU14.6 | <i>Saccharopolyspora cebuensis</i> | - | - | + | - | - | + | - |
| | BU16.8 | <i>Salinicola salarius</i> | - | + | - | - | - | - | - |
| | SA7.6 | <i>Acinetobacter soli</i> | - | - | - | - | + | - | - |
| | SA7.7 | <i>Micrococcus yunnanensis</i> | - | - | + | - | - | - | - |
| Total | 156 | 23 | 4 | 10 | 6 | 0 | 4 | 2 | 0 |

Note: **A:** *S. aureus*; **B:** *E. coli*; **C:** *P. aeruginosa*; **D:** *S. saprophyticus*; **E:** *A. baumannii*; **F:** *K. pneumonia*; **G:** *C. albicans*.

16S rDNA gene and phylogenetic analysis

The 16S rRNA gene sequencing analyses showed that these 23 isolates could be assigned to 14 different species within the three phyla: Actinobacteria (*Streptomyces zhaozhouensis*, *Nocardiopsis salina*, *Micrococcus endophyticus*, *Brevibacterium casei*, *Micrococcus yunnanensis*, *Saccharopolyspora coralli*, *Kocuria salina*), Firmicutes (*Bacillus paramycoides*, *Virgibacillus salaries*, *Oceanobacillus iheyensis*, *Marinococcus halophilus*) and Proteobacteria (*Vibrio alginolyticus*, *Acinetobacter soli*, *Salinicola salarius*). Six of the 23 isolates (26.08%) were members of the genus *Marinecoccus*, followed by *Nocardiopsis* with five isolates (21.7%). The remaining 12 of the 23 isolates (52.1%) were a genus of the *Streptomyces*, *Brevibacterium*, *Saccharopolyspora*, *Bacillus*, *Virgibacillus*, *Oceanobacillus*, *Vibrio*, *Acinetobacter*, *Salinicola*, *Marinecoccus*, *Pseudomonas* and *Kocuria*. Identification of pairwise 16S rRNA gene similarities was analysed by using NCBI-BLAST homology. The PAUP v.05 (Swofford 1998) and CLUSTAL_X (Thompson et al. 1997) were used to construct phylogenetic trees (Fig. 3).

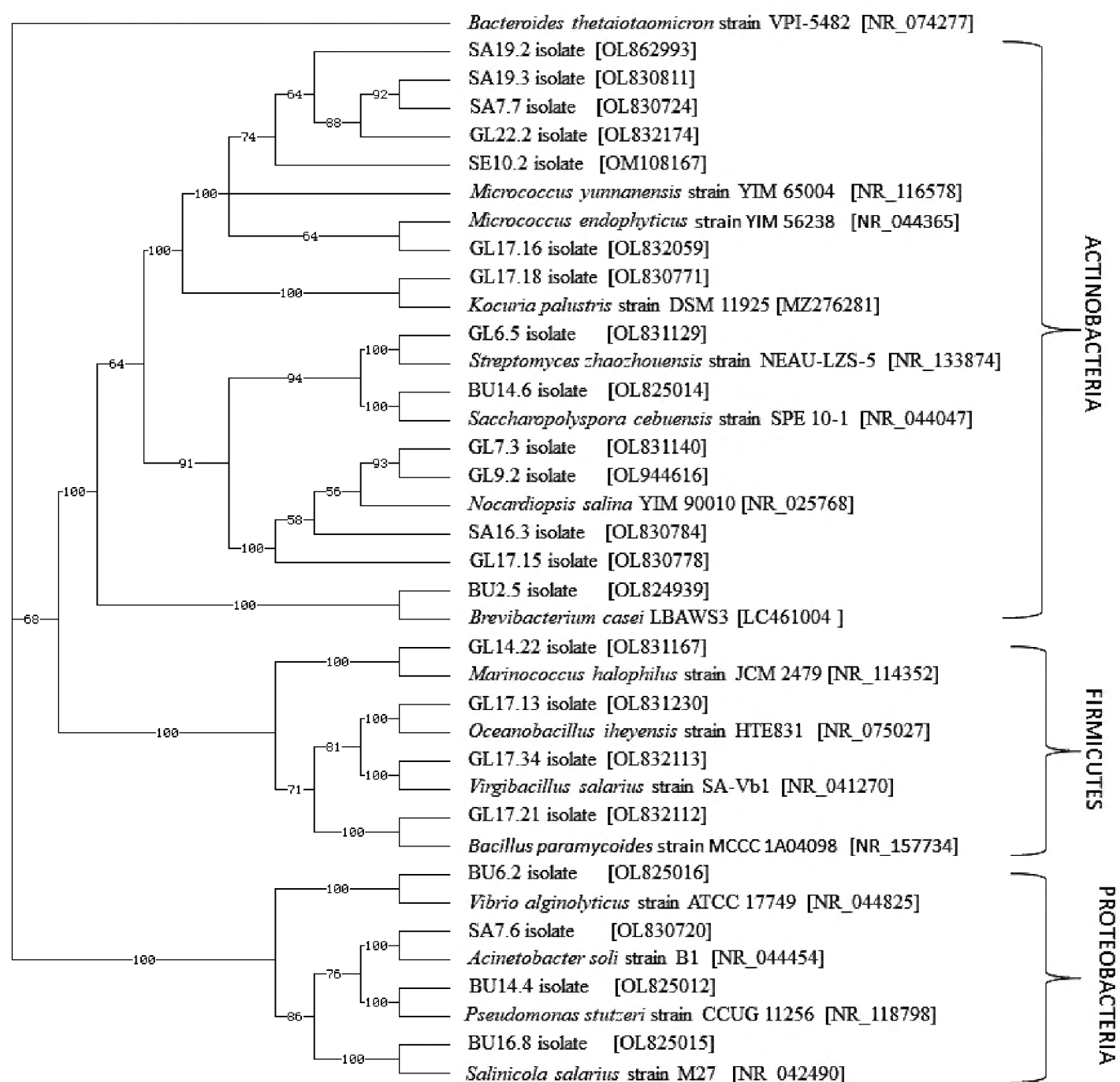


Figure 3. Phylogenetic-tree of Gorgonian-associated bacteria with antipathogen properties.

Distribution of bacteria with antibacterial activity

The distribution, Diversity Index, Species Richness and Evenness of antibacterial isolates are presented in Table 3, Figs 4 and 5. The number of antibacterial strains isolated from MPA and non-MPA areas was 17 (73.9%) and six isolates (26.08%), respectively. Amongst these anti-bacterial isolates, *Micrococcus* had the highest proportion (26.08%), followed by *Nocardiopsis* (21.7%), *Streptomyces* (4.34%), *Brevibacterium* (4.34%), *Saccharopolyspora* (4.34%), *Bacillus* (4.34%), *Virgibacillus* (4.34%), *Oceanobacillus* (4.34%), *Vibrio* (4.34%), *Acinetobacter* (4.34%), *Salinicola* (4.34%), *Marinecoccus* (4.34%), *Pseudomonas* (4.34%) and *Kocuria* isolates (4.34%).

Furthermore, 43.4% (10 isolates) of antibacterial isolates displayed strong activity against *E. coli* and about 29.09% (six isolates) of antibacterial isolates displayed activity against the pathogenic bacteria *P. aeruginosa*. Four bacterial isolates (17.4%) showed activity against *S. aureus* and *A. baumannii*. No bacterial isolate displayed activity against *S. saprophyticus* and *C. albicans*.

Comparison of bacterial communities in the MPA and non-MPA and amongst Gorgonian genera were analysed by the Bray-Curtis formula. The analysis results demonstrated that the bacterial community's dissimilarity between MPA and non-MPA and amongst Gorgonian genera were significantly different (Tables 4 and 5). The results indicated that the bacterial communities varied significantly.

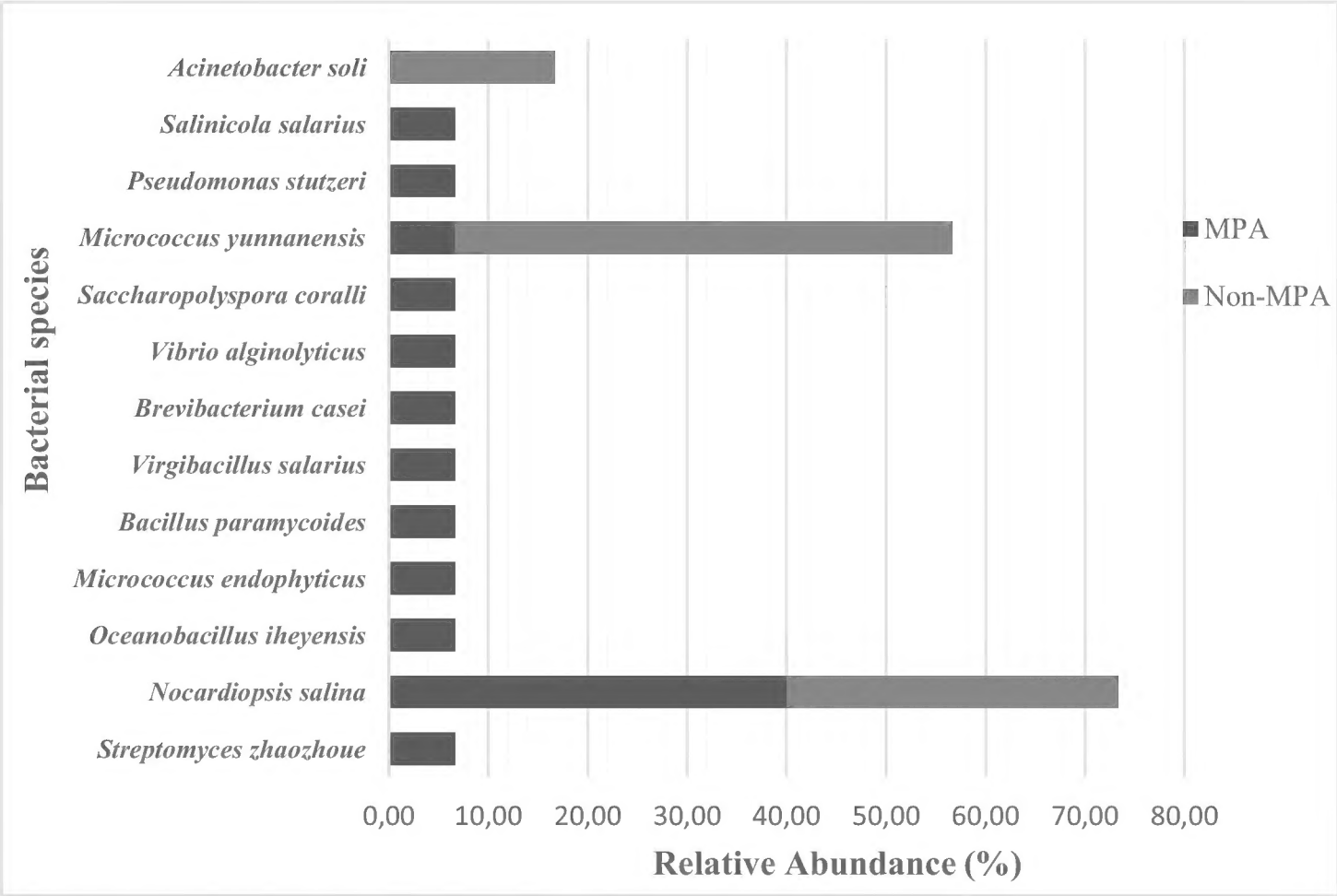


Figure 4. Relative Abundance (RA) of Gorgonian-associated bacteria.

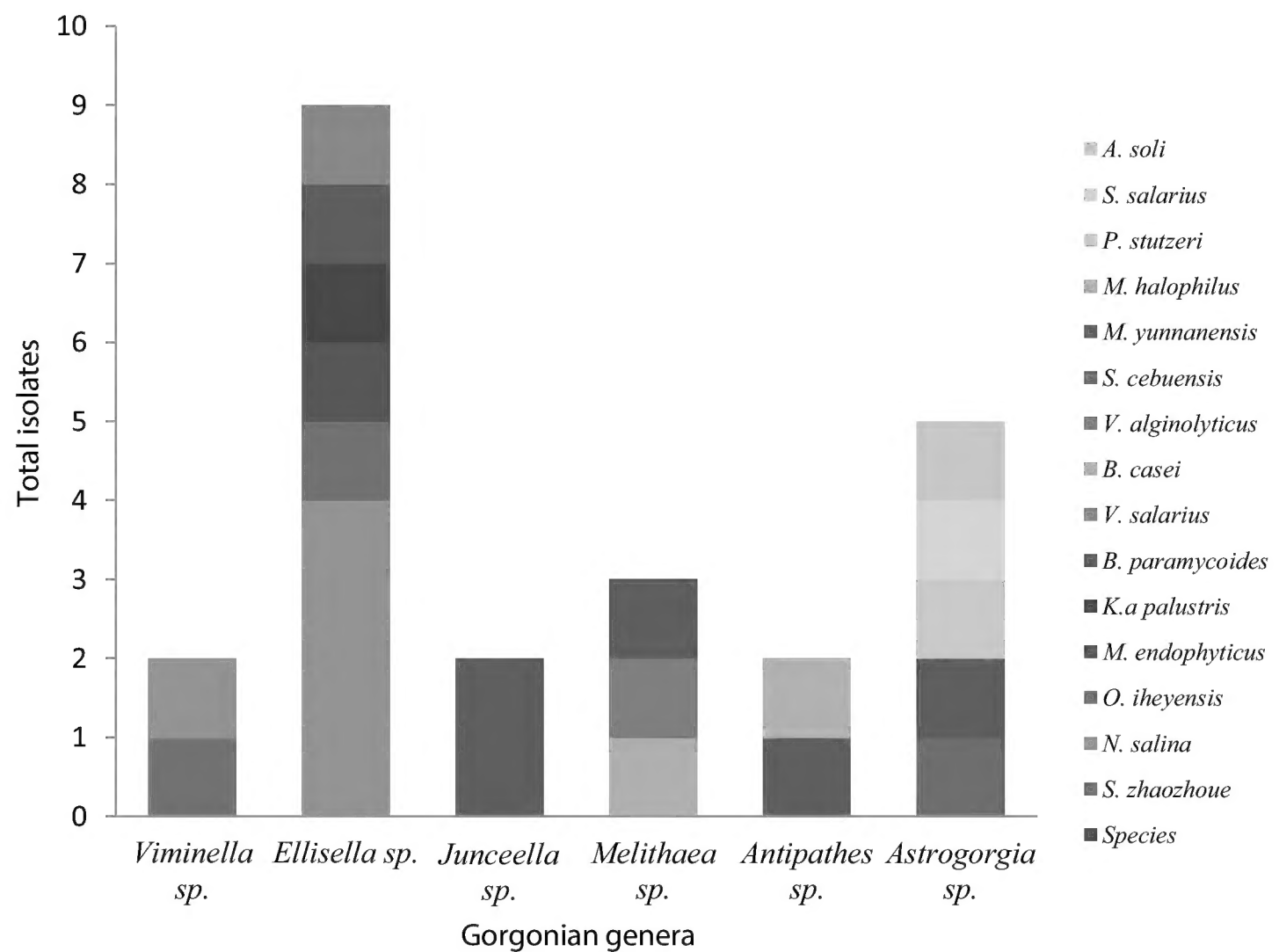


Figure 5. The abundance of antibacterial isolates on Gorgonian genera.

Table 3. Relative Abundance, Species Diversity and Evenness of antipathogenic isolates.

| Phylum | Species | MPA | | Non-MPA | |
|------------------|----------------------------------|------|-------|---------|-------|
| | | N | RA | N | RA |
| Actinobacteria | <i>Streptomyces zhaozhou</i> | 1 | 6.67 | 0 | 0 |
| | <i>Nocardiopsis salina</i> | 5 | 40.00 | 2 | 33.33 |
| | <i>Micrococcus endophyticus</i> | 1 | 6.67 | 0 | 0 |
| | <i>Brevibacterium casei</i> | 1 | 6.67 | 0 | 0 |
| | <i>Micrococcus yunnanensis</i> | 1 | 6.67 | 3 | 50.00 |
| | <i>Kocuria palustris</i> | 1 | 6.67 | 0 | 0 |
| | <i>Saccharopolyspora coralli</i> | 1 | 6.67 | 0 | 0 |
| Firmicutes | <i>Bacillus paramycoides</i> | 1 | 6.67 | 0 | 0 |
| | <i>Virgibacillus salarius</i> | 1 | 6.67 | 0 | 0 |
| | <i>Oceanobacillus iheyensis</i> | 1 | 6.67 | 0 | 0 |
| Proteobacteria | <i>Vibrio alginolyticus</i> | 1 | 6.67 | 0 | 0 |
| | <i>Pseudomonas stutzeri</i> | 1 | 6.67 | 0 | 0 |
| | <i>Salinicola salarius</i> | 1 | 6.67 | 0 | 0 |
| | <i>Acinetobacter soli</i> | 0 | 6.67 | 1 | 16.66 |
| Total | | 17 | | 6 | |
| Species Richness | | 13 | | 3 | |
| Diversity Index | | 1.99 | | 1.01 | |
| Evenness | | 0.86 | | 0.92 | |

Note: Number of individuals (N); Relative abundance, % (RA).

Table 4. The dissimilarity of antimicrobial strains between MPA and non-MPA.

| Sampling sites | MPA | Non-MPA |
|----------------|--------|---------|
| MPA | – | 73.91% |
| Non-MPA | 73.91% | – |

Table 5. The dissimilarity of antimicrobial communities amongst Gorgonian genera.

| | A | B | C | D | E | F |
|---|--------|--------|--------|--------|--------|--------|
| A | – | 81.82% | 100% | 100% | 100% | 100% |
| B | 81.82% | – | 100% | 100% | 100% | 100% |
| C | 100% | 100% | – | 60% | 50% | 77.43% |
| D | 100% | 100% | 60% | – | 60% | 71.43% |
| E | 100% | 100% | 50% | 60% | – | 77.43% |
| F | 100% | 100% | 77.43% | 77.43% | 77.43% | – |

Note: **A:** *Viminella* sp.; **B:** *Ellisella* sp.; **C:** *Antipathes* sp.; **D:** *Melithaea* sp.; **E:** *Astrogorgia* sp.; **F:** *Junceella* sp.

Discussion

Gorgonian is a benthic community that harbours distinct microbial symbionts in seawater (van de Water et al. 2018). Their populations have suffered from mass mortality events related to anthropogenic disturbances. Changes in environmental conditions due to anthropogenic and natural disturbances can alter the microbial composition (McCauley et al. 2020). In this study, the six species of Gorgonian- (*Viminella* sp., *Ellisella* sp., *Antipathes* sp., *Melithaea* sp., *Astrogorgia* sp. and *Junceella* sp.) associated bacteria from the MPA and non-MPA zones of Karimunjawa, Java Sea were investigated for their diversity and antibacterial activity. It is well-known that marine invertebrate-associated bacteria were abundant and prolific in novel active compound production (Bibi et al. 2020; Varijakzhan et al. 2021). The 156 Gorgonian-associated bacteria were assayed for their prospective antipathogenic compounds against seven UTI pathogens, including *S. aureus*, *E. coli*, *P. aeruginosa*, *S. saprophyticus*, *A. baumannii*, *K. pneumonia* and *C. albicans*. The results showed that 23 isolates demonstrated inhibition of the growth of UTI pathogens (Table 1 and Table 2). Their genomic DNA was extracted and sequenced for molecular identification. The 23 anti-bacterial isolates were identified as 14 genera, including *Micrococcus*, *Nocardiopsis*, *Streptomyces*, *Brevibacterium*, *Saccharopolyspora*, *Bacillus*, *Virgibacillus*, *Oceanobacillus*, *Vibrio*, *Acinetobacter*, *Salinicola*, *Marinecoccus*, *Pseudomonas* and *Kocuria*. Phylogenetic analysis showed that the 23 isolates analysed in the present study could be classified into three clades (I–III): Clade I consisted of the isolates of phyla Actinomycetes; Group II of the isolates is Firmicutes; Group III of the isolates consisted of Proteobacteria (Table 2 and Fig. 3). Some previous studies have identified the Gorgonian-associated bacteria with antimicrobial activity. Zhang et al. (2013) demonstrated that *Streptomyces* and the *Micromonospora* genus dominated Gorgonian-associated antibacterial isolates from five species of Gorgonian of the South China Sea. Jiang et al. (2013) reported seven

genera, including *Bacillus*, *Staphylococcus*, *Halobacillus*, *Geobacillus*, *Jeotgalicoccus*, *Psychrobacter* and *Vibrio*, isolated from the four species of South China Sea Gorgonians. In addition, the *Bacillus* genus was the most diverse and displayed antibacterial activities. These results indicate that each Gorgonian species has varying numbers and types of bacterial groups. On the contrary, Gray et al. (2011) showed that the same Gorgonian corals at different locations have different kinds of bacteria. So far, little is known about how the diversity of Gorgonian-associated bacteria diverges amongst Gorgonian coral species, particularly about how environmental factors influence this relationship. Hence, a study of Gorgonian-bacterial diversity is urgently needed to help understand more about microbial diversity-function relationships.

During the last decade, MPAs have been used to conserve marine resources worldwide, although little is known about their effectiveness and success. A comparison of antibacterial composition between MPA and non-MPA showed that 17 of 92 (18.48%) and six of 64 (9.37%) bacterial isolates from MPA and non-MPA, respectively, exhibited antimicrobial activity in at least one of the UTIs pathogens. The analyses results also showed the Relative Abundance, Species Richness and Diversity Index of antibacterial isolates in the MPA were higher than those of non-MPA zones. (Table 3, Figs 4 and 5). Bourne et al. (2013) stated that the Species Richness, Evenness and phylogenetic diversity of invertebrate-associated microbiomes were not influenced by the bacterial composition. However, these results demonstrated that Gorgonian octocoral species in the MPA region harbour varied bacteria and we propose that many Gorgonian-associated bacteria have the prospective for advancing broad-spectrum antibiotics. Our results have significant implications for PA management being effectively undertaken and thus contributing to biodiversity protection.

The MPA and non-MPA antibacterial isolates were dominated by the same phyla Actinobacteria (Table 3, Figs 4 and 5); however, the Relative Abundance, Species Richness and Diversity Index exhibited significant differences. *Micrococcus* genera dominated both the MPA and the non-MPA samples. Some previous studies reported the diversity of Gorgonian coral-associated bacteria. This study found that the number and genera of bacterial isolates from MPA and non-MPA were significantly different (Tables 4 and 5). The abundance and diversity of antimicrobial isolates in MPA were higher than in non-MPA. On the contrary, Nogales et al. (2011) stated that microbial communities increase diversity and variability in polluted areas. Due to these differences, it is obvious that little is known regarding the effect of anthropogenic activities on the composition of microbial communities. Even the anthropogenic stress covered in this study only modified the environmental conditions by zoning marine areas; however, it is a complex situation.

The Gorgonian coral *Ellisella* sp. concealed the most antibacterial activities of isolates and the highest diversity of antibacterial activity genera (Figs 4 and 5). At the same time, Bayer et al. (2013) reported that Gorgonian coral *Eunicella cavolini* was dominated by the genus *Endozoicomonas* sp. It seems that the totals and kinds of bacterial groups varied amongst Gorgonian species. In this study, only about 15% (23 isolates) of Gorgonian-associated bacteria were active against at least one of the

pathogenic UTIs, which means that only a few Gorgonian-associated bacteria produced active antimicrobial compounds. Recently, marine invertebrate-associated microorganisms have become a potential source of new active compounds. Several recent studies have shown that bacteria isolated from tunicates, nudibranchs, sponges and soft corals produce promising antibacterial active compounds (Putra et al. 2016; Cita et al. 2017; Ayuningrum et al. 2019; Kristiana et al. 2019). This study demonstrated that culturable Gorgonian-associated bacteria could produce antibiotics and inhibit the growth of UTI nosocomial pathogenic bacteria. The diversity and abundance of Gorgonian-associated bacteria with antibacterial properties were higher in MPA than in non-MPA zones, indicating effective and efficient management in biodiversity protection. Maintenance of trophic structure and diversity of functions are necessary efforts that must be undertaken as a management priority to enable ecosystem resilience.

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Supplementary material I

Tables S1–S3

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Data type: Dataset on occurrences, morphological and image.

Explanation note: In this study, bacterial communities associated with six species of gorgonian, *Viminella* sp., *Ellisella* sp., *Antipathes* sp., *Melithaea* sp., *Astrogorgia* sp, and *Junceella* sp. from both the Marine Protected Area (MPA) and non-Marine Protected Area (non-MPA) zones were screened for their antipathogenic potential against Urinary Tract Infections (UTIs) pathogens. The selected bacterial isolates were identified and compared for their abundance and diversity between the two zones. 156 bacterial strains were assayed for their prospective antipathogenic compounds against seven UTI pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus saprophyticus*, *Acinetobacter baumannii*, *Klebsiella pneumonia*, *Candida albicans*.

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